Investigating the role of MUC13 in *Clostridioides difficile* Infection

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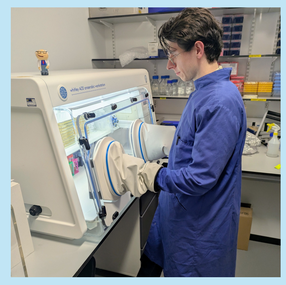


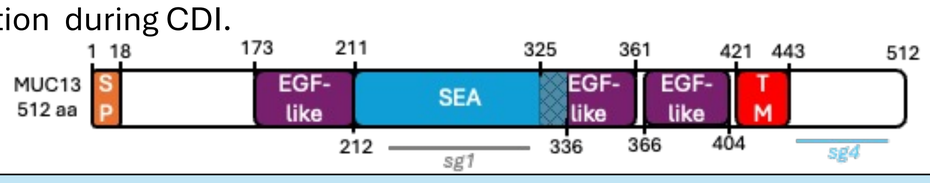
Introduction

The mucus layer forms an important barrier between the intestinal lumen and intestinal epithelial cells (IECs), preventing bacteria from establishing infections in the gut. Barrier disruption is a key factor in *Clostridioides difficile* infection (CDI).

The mucus layer in the human colon at the site of CDI is made up of twelve mucin proteins.

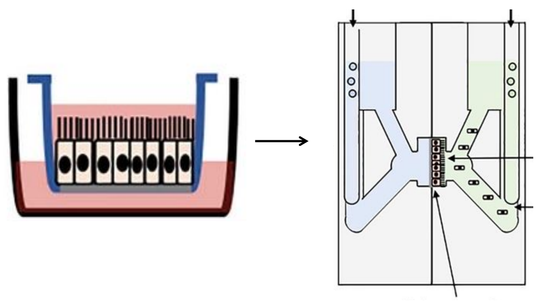
Previous studies have shown the importance of mucins in diverse bacterial infections including *Salmonella* Typhimurium and *Helicobacter pylori* infections.

We have previously shown that MUC13 is upregulated during CDI. However, its functional role in *C. difficile* colonisation of the gut and barrier disruption is not known. We hypothesise that MUC13 plays an important role in early *C. difficile* colonisation during CDI.

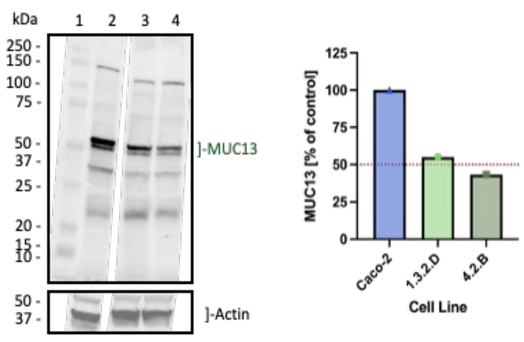


Methods

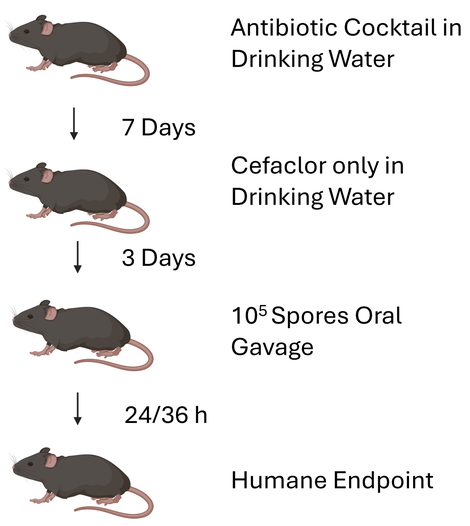
- Polarised Caco-2 epithelial monolayers were cultured in a vertical diffusion system (VDC). In vitro infection assays used the strain R20291 and an MOI of 100.



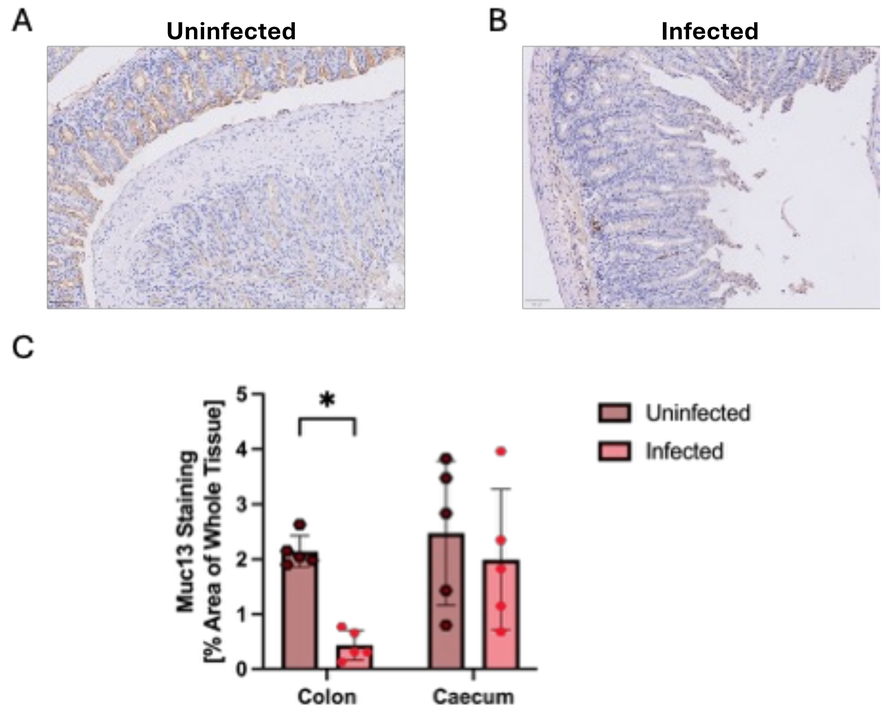
- MUC13 knockdown cell lines were generated with the PX459 plasmid system using gRNA sequences designed from the CHOPCHOP online tool. Successful knockdown was verified using immunoblotting and TIDE analysis.



- *For in vivo* infection assays (Monash University), mice were pre-treated with a drinking water antibiotic regime consisting of kanamycin, gentamicin, colistin, metronidazole, vancomycin and cefaclor and then challenged with 10^5 spores by oral gavage. An experimental endpoint was reached at 24 or 36 hours post infection (p.i). Strains M7404 and DLL3109 were used.

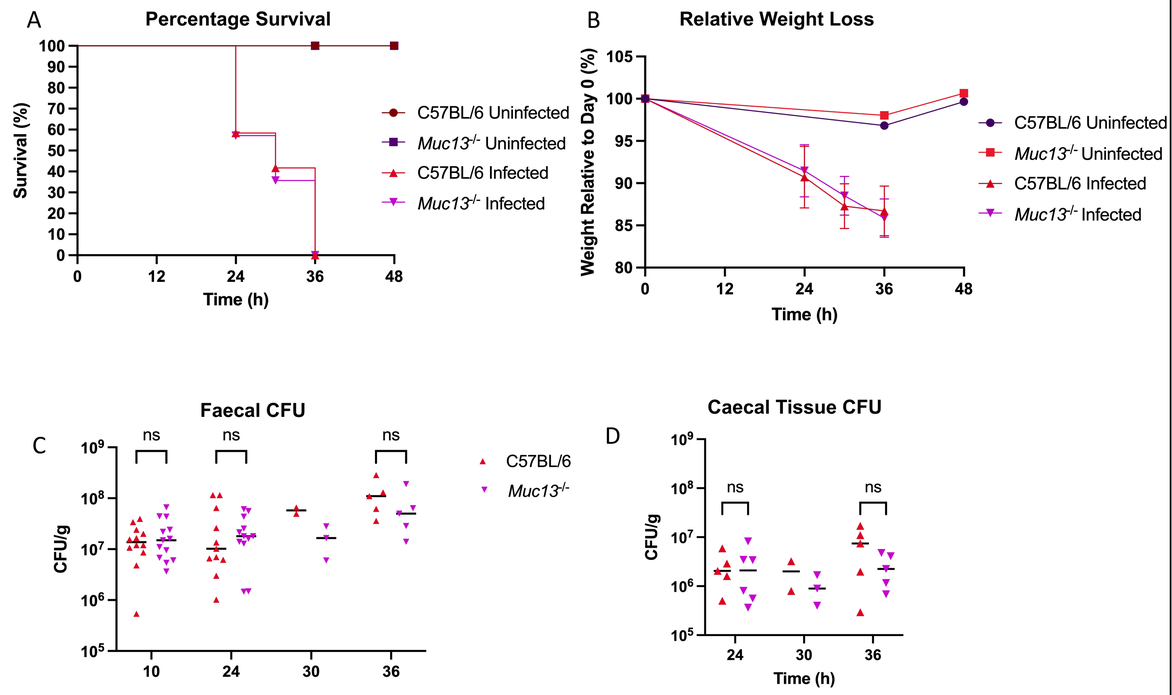


MUC13 levels in caecal and colonic tissue following *C. difficile* infection in vivo.



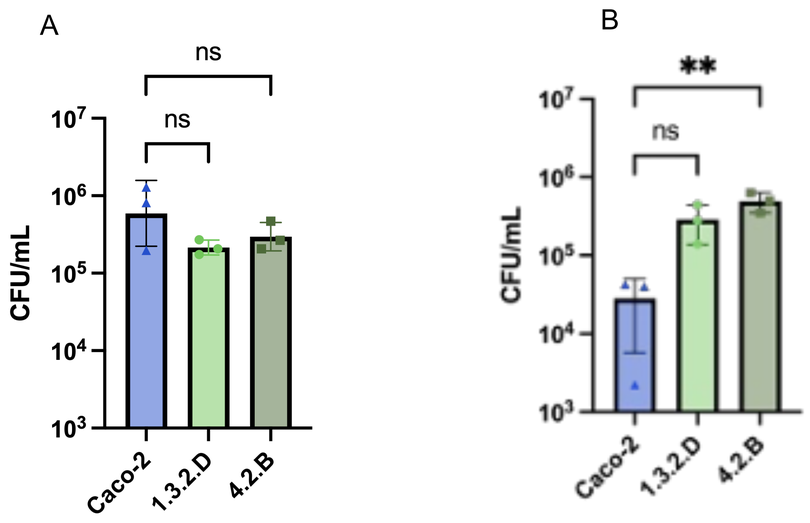
C57BL/6 mice were infected with *C. difficile* strain DLL3109. (A and B) Caecal tissues were probed for MUC13 by immunohistochemistry. (C) Quantification of MUC13-positive area.

Muc13$^{-/-}$ mice show no increased susceptibility to *C. difficile* infection.



C57BL/6 or *Muc13*$^{-/-}$ mice were infected with 10^5 spores of *C. difficile* strain M7404 as described in methods. (A) Kaplan-Meier survival curve. Mice euthanised after losing >10% body weight in 24-hours or >15% thereafter. (B) Weight loss relative to day zero for infected or uninfected groups. (C+D) Total CFU recovered from faecal (C) and homogenised caecal (D) samples plated onto germinating media selective for *C. difficile*.

MUC13 contributes to infection in an *in vitro* gut model.



Caco-2 cells (unmodified or MUC13 knockdown) were challenged with *C. difficile* R20291 wild-type at an MOI of 100:1 for (A) 6 hours on polarised epithelial monolayers or (B) 24 hours in an *in vitro* vertical diffusion chamber (VDC) model. ns = not significant, * = *p*-value < 0.05. Bacteria were cultured and CFU counts prepared in a **Whitley A35 Workstation.**

Muc13$^{-/-}$ mice showed similar histopathological response to *C. difficile* infection to WT.

WT C57BL/6 or *Muc13*$^{-/-}$ mice were infected with *C. difficile* strain M7404. Caecal tissues were sectioned and stained for histopathological analysis. Representative H&E-stained microscopic images at x20 magnification.

Summary

- MUC13 may contribute to early bacterial survival within a gut model in vitro.
- Despite MUC13 levels being altered during CDI, in mice, it is not a major determinant of disease outcome following acute infection.
- It is possible that there is redundancy in host mucin production to protect against a broad range of gut pathogens.
- Further work should look to contextualise the role of MUC13 in CDI with other mucins.